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14. ABSTRACT Prostate cancer (PC) is the second leading cause of male U.S. cancer deaths, with African-Americans having the highest rate of PC mortality worldwide, as well as more abnormal results from screening tests that correlate with current or eventual PC. A 5-year prospective NIH-funded clinic-based study investigated whether prostate-specific antigen (PSA) and digital rectal exam (DRE) screening indicators of PC risk in 500 African-American men 50 to 70 years of age who underwent PC screening in Oakland, CA (East Bay San Francisco area), were associated with estimated dietary exposures to 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), which forms when meat is overcooked. The DOD-funded study expands that NIH-funded work by adding a new %-free-PSA test for 310 (108 from the NIH-funded study, plus 202 additional) men, results of which will be compared with PSA/ DRE results and PhIP exposures estimated by dietary interviews. For 392 men studied under the NIH protocol, an odds ratio (95% CL) of 32 (3.2, 720) for highly elevated PSA (=20 ng/mL) was observed in the highest 15% vs. the lower 50% of estimated daily PhIP intakes. As of 09-01-07, a total of 310 men completed participation using the expanded protocol, for a combined total of 702 men. For the final analysis of all 702 men, the corresponding OR was found to be 10 (2.9, 58). This study will help define the potential value of improved screening and dietary/behavioral intervention to reduce PC risk.					
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## Introduction

### PhIP is a Dietary Carcinogen that May Pose Heightened Risk to African-American Men

African American (AA) men, who compared to Caucasians die nearly twice as much from prostate cancer (PC), also take in about twice as much of the predominant U.S. dietary heterocyclic amine, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) (Bogen and Keating, 2001), which occurs primarily in well-cooked chicken and beef. Heterocyclic amines (HAs) are potent mutagens formed in meats, chicken and fish as it is cooked to higher-doneness levels by heat-intensive cooking methods (Thompson *et al.*, 1987; Keating *et al.*, 1999, 2000). HAs also cause cancer at a variety of sites in multiple bioassay animal species/strains/sexes, as well as at multiple sites within many of species/strains/sexes tested (Bogen, 1994). A predominant HA found in cooked and particularly in well-done chicken and beef is 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) (Felton *et al.*, 1984, 1986; Felton and Knize, 1990a-b; Sinha *et al.*, 1995). Dietary exposure to PhIP has been shown to induce colon, intestinal and mammary adenocarcinomas in rats (Ohgaki *et al.*, 1986; Ochiai *et al.*, 1991; Ito *et al.*, 1991; Ito *et al.*, 1997; Ghoshal *et al.*, 1994), as well as prostate cancer in rats (Shirai *et al.*, 1997, 1999). HAs are metabolically activated by P450 and N-acetyltransferase enzymes to activated forms that bind covalently to (among other targets) DNA in tissues (including in prostate) where HAs induce cancer in rats (Thorgeirsson *et al.*, 1983; Thorgeirsson, 1984; Rosenkranz and Mermeistein, 1985; Sato *et al.*, 1986; Kato and Yamazoe, 1987; Snyderwine and Battula, 1989; McManus *et al.*, 1990; Turesky *et al.*, 1991; Davis *et al.*, 1993; Kaderlik *et al.*, 1994a-b; Takahashi *et al.*, 1998; Schut and Snyderwine, 1999; Gooderham *et al.*, 2002). In male *lacI* transgenic rats, a diet containing 200 ppm PhIP was shown to be highly mutagenic in prostate tissue (Stuart *et al.*, 2000). In cultured human prostate tissue and primary prostate cells, PhIP is metabolically activated to mutagenic forms that covalently bind to and damage DNA (Williams *et al.*, 2000; Lawson and Kolar, 2002; Martin *et al.*, 2002; Di Paolo *et al.*, 2005).

### PC and African-American men

In the U.S., prostate cancer (PC) is a leading cause of cancer death among men, with African Americans having the highest age-specific prostate cancer rate in the world, and a >2-fold higher rate of mortality for PC than white men in the U.S. (Miller *et al.*, 1996; Robbins *et al.*, 1998; Hsing *et al.*, 2000). Although family (particularly father/brother) history of PC is clearly linked to substantially elevated PC risk (Schuurman *et al.*, 1999; Hemminki and Czene, 2002; Zeegers *et al.*, 2003; Hemminki and Chen, 2005) and a human-PC-specific chromosome translocation has been identified (Tomlins *et al.*, 2005), there is (as for most other cancers) no evidence for a predominant heritable factor for PC. Racial-ethnic differences in levels of testosterone-related hormones and related genetic controls on hormone-induced prostate growth have been hypothesized to explain or contribute to corresponding racial-ethnic differences in PC risk, but their role remains unclear (Ross *et al.*, 1998; Pettaway, 1999; Hsing, 2001). Even if hormonally mediated background processes do affect PC risk, dietary exposures to genotoxic HA

carcinogens may act independently or interact synergistically with such hormonal processes to further modify PC risk.

#### HA, Meat, Other Dietary Factors, and PC Risk

Substantial geographical variations in PC incidence indicate the importance of one or more dietary or other environmental factors (Minami *et al.*, 1993; Mettlin, 1997; Angwafo, 1999). Dietary intake of animal or saturated fat is the environmental factor most consistently linked to significantly increased PC risk in previous studies, particularly among African-American men, though these associations appear too weak to explain more than a small fraction of observed racial-ethnic differences in PC risk (Whittemore *et al.*, 1995; Hayes *et al.*, 1999; Kolonel *et al.*, 1999; Daniels *et al.*, 2004), as also appears to be the case for other environmental/dietary factors examined such as calcium, cruciferous vegetables, vitamin D, UV from sunlight, lycopene, and body size (Giovannucci *et al.*, 1997, 1998; Cohen *et al.*, 2000; Chan and Giovannucci, 2001a-b; Kristal and Lampe, 2002; Bodiwala *et al.*, 2003). Because cooked-meat intake is positively associated with total saturated-fat intake (USDA, 1998), previous studies that focused on animal or saturated fat *per se* could have estimated effects due largely or entirely to meat-related HA intakes.

Consumption of cooked meats and associated HAs have been linked to increased risks of colorectal adenoma/adenocarcinoma and of cancer of the stomach, breast, lung and prostate (Mills *et al.*, 1989; Shiffman and Felton, 1990; Gerhardsson de Verdier *et al.*, 1991; Talamini *et al.*, 1992; Lang *et al.*, 1994; De Stefani *et al.*, 1995; Ewings and Bowie, 1996; Probst-Hensch *et al.*, 1997; Ward *et al.*, 1997; Kampman *et al.*, 1999; Norrish *et al.*, 1999; Sinha *et al.*, 1998a, 1999a-b; Zheng *et al.*, 1998, 1999; Murtaugh *et al.*, 2004), while fewer studies found no such associations (Lyon and Mahoney, 1988; Muscat and Wynder, 1994; Augustsson *et al.*, 1999). Positive studies include those in which HA exposure was quantified adjusting for factors expected to determine HA intake—namely, meat type, consumption frequency, cooking method, and cooking doneness. A potential link between PhIP intake, in particular, and the elevated risk of PC experienced by African-American compared to Caucasian men is suggested by relatively greater levels of PhIP and its metabolites detected in urine sampled from the latter vs. the former group (Kidd *et al.*, 1999), although urine analysis only provides a measure of PhIP exposure within 12-24 hours prior to sampling (Malfatti *et al.*, 1999). Such a link is also supported by studies using meat- and cooking-method-specific HA-concentration estimates from multi-laboratory sets of experimental cooking data (Keating and Bogen, 2001) to assess intakes of PhIP and other HAs by >20,000 U.S. (including >3,000 African-American) participants in the nationwide, stratified, random-sample U.S. Continuing Survey of Food Intakes by Individuals (CSFII) (USDA, 1993, 1998). Analyzed by age-, sex, and race/ethnicity, these HA-exposure assessments found at all ages that African-American males consume on average at least twice as much PhIP (and total HA) per kg body weight per day as do U.S. Caucasian males, and that for both groups there is a significant positive (albeit small) correlation between estimated mean intakes of PhIP and those of saturated or total fat (Keating and Bogen, 2001; Bogen and Keating, 2001).

Although the study comparing HA intake (primarily from cooked lamb) and PC risk in New Zealand men by Norrish *et al.* (1999) found no significant PhIP-related associations involving PC, that study did report a significant association between PC risk and consumption of beefsteak by higher cooking-doneness category (2-sided  $p_{\text{trend}} = 0.008$ ). Several aspects of that study suggest it may have had limited power to assess potential associations between PC and HA or PhIP intake in the U.S. The incidence of PC in New Zealand is only half that of Caucasian men in the U.S. (Hsing *et al.* 2000). Average meat (primarily lamb) consumption by subjects in the Norrish *et al.* study (~150 g/d) was well below that of U.S. Caucasian men (260 g/d) and very well below that of African-American men (304 g/d) (USDA, 1998; Table 10A). Moreover, the *minimum* PhIP intake (224 ng/d) in the *top* exposure quartile of Norrish *et al.* study subjects was well below the estimated *average* PhIP intakes by U.S. Caucasian (390 ng/d) and by African-American (600 ng/d) men (Bogen and Keating 2001).

A randomized, controlled multisite prospective study that ascertained 1,338 PC cases between 1993 and 2001 among 29,361 (including 3.3% African American) men of age 55-74 years in 10 U.S. cities was conducted specifically to determine whether meat intake or meat-related mutagens, including PhIP, was associated with increased PC risk (Cross *et al.*, 2005). In this study, intakes of meat, PhIP, two other heterocyclic amines, and meat-associated Ames-assay mutagenic yield were all estimated using FFQs that were self-administered, but otherwise very similar to those currently administered (by in-person interview) in the clinical study which Project 2 of this application would extend. More than 10 g/day vs. no intake of very well done meat was found to be associated with a 1.7-fold increased risk (95% CI: 1.19-2.40), and the highest vs. lowest PhIP quintile with a 1.3-fold increased risk (95% CI: 1.01-1.61), of PC incidence (Cross *et al.*, 2005). Other intakes were not found to be associated with increased PC risk. No separate analysis was done of study results by race-ethnicity was not reported, nor were genotype or other (e.g., dietary) factors considered that are known to affect PhIP metabolism.

### PC Screening and PC

Periodic screening for plasma levels of Prostate-Specific Antigen (PSA) is useful for early detection of PC as well as benign prostatic hyperplasia (BPH) because PSA levels in serum are positively associated with age, prostate volume, and prostatic neoplastic disease (Carter *et al.*, 1992; Oesterling *et al.*, 1993; Etzioni *et al.*, 1999). Significantly higher PSA levels are found in African-Americans than in whites, even after adjustment for age and prostate volume (in men without PC) and for PC grade and stage (in men with PC) (Abdalla *et al.*, 1998a-b; Vijayakumar *et al.*, 1998), due evidently to greater PSA secretion per unit prostate volume by African-American men (Fowler *et al.*, 1999). Although a substantial fraction of “slightly” elevated PSA levels (between 4 and 10 ng/mL) is attributable to BPH or infection, “highly elevated” PSA levels ( $\geq 20$  ng/mL) typically indicate a strong likelihood of localized or metastatic PC, with 80 to 90% positive predictivity and >99% specificity (Mänttinen *et al.*, 2001; Gerstenbluth *et al.*, 2002; Smith *et al.*, 2004). Likewise, a PSA measure < 4.0 ng/mL often considered within the “normal” range is actually associated with about at 15% of later PC diagnosis (Thompson *et al.*, 2004).

## Report Body

**Objective/Hypotheses:** The study goal is to broaden the scope and power of a ground-breaking study of potential association between dietary HA exposure and screening indicators of PC risk in African-American men by adding a newer %fPSA test to the PSA/DRE protocol now being used. We hypothesize 1) that the added %fPSA test will increase PC detection in our study population, and 2) that the combined screening, follow-up diagnostic and dietary survey data obtained will reveal a positive association between estimated HA intake and screening and diagnostic indicators of increased PC risk in our African American study population.

**Specific aims:** Our aims are to 1) Add a newer %fPSA test to PSA and DRE screens being done in a current study of potential associations between HA and PC in Oakland, California, area African-American men. 2) Assess potential increased rate of PC identified by including the %fPSA test with PSA and DRE results in light of clinical follow-up diagnoses obtained. 3) Use PSA-related and DRE test results, together with corresponding follow-up diagnostic and dietary survey data, to assess the potential association of HA-related exposure factors and increased PC risk in African-Americans.

**Study Design:** This is a prospective, clinic-based screening study. For aim 1, 392 participants were solicited from an already-established network of churches, clinics and additional African-American community groups in the Oakland, CA, area. The DOD-funded work continues this effort, adding the new %fPSA biomarker. Detailed data on diet and meat consumption continue to be obtained by in-person interviews using established questionnaires, each followed by PSA-related and DRE screening tests, and follow-up diagnosis—a study design that avoids potential bias due to prior participant/investigator knowledge of PC status. Aims 2 and 3 are being accomplished by statistical data analysis, with aim 3 to be supported by incorporation of similar (other than %fPSA) data obtained for the 392 participants already obtained through the NIH-funded study that ended in 2006, for a total combined study size of 702 men. Our focus on African-Americans provides needed study power, in view of the greater PC risk faced by this specific group.

## **Progress in Relation to Work Plan**

### **Work Plan Summary**

A total of 392 participants were accrued previously in the NIH-funded study through December 2004 using dietary interviews, a digital rectal exam (DRE), and a standard prostate serum antigen (PSA) blood test. The DOD Prostate Cancer Research Program support has added a second PSA-related test—the “percent-free PSA” (%fPSA) test—that was applied to 310 additional study participants through September of 2007, for whom blood samples were drawn for both a PSA and a %fPSA test and the dietary interview and DRE done, all at the study clinic in Oakland on the same day for each participant.

### **Delayed Receipt of Funds and HSRRB Approval of Human Subjects Protocol**

Initiation of the study was delayed due to delayed receipt of DOD funds by Lawrence Livermore National Laboratory (LLNL). Due to contract language negotiations, funds arrived at LLNL approximately four months after the official start date of the project (Jan. 5, 2005). A further start-up delay occurred due to delayed receipt of U.S. Army Human Subjects Research Review Board (HSRRB) approval of the study human subjects protocol, which had already received approval by the other three Institutional Review Boards (IRBs) involved in this study (those of LLNL, the University of California San Francisco Medical School, and the Summit Alta Bates medical Center in Oakland, CA). A draft human subjects protocol was submitted to the HSRRB in December of 2004, but HSRRB approval was not obtained until mid-May of 2005.

The delayed start-up and extended IRB-related documentation required to start this project caused small, unanticipated shifts in LLNL labor relative to the original budget plan. However, the project is currently planned for completion per the original project schedule.

Study Progress (indicated below in bold)

Task 1. Add %fPSA test to the standard PSA blood test and DRE being done in a current study protocol to assess potential HA/PC associations in Oakland-area African American men (Months 1-36). **Completed as of September 1, 2007.**

1.A. Obtain IRB approvals for modified study protocol (Months 1-3). Accomplishment of this task was facilitated by the established protocol used in the prior NIH-funded study; approvals were obtained for a modification of this protocol to add the %fPSA test to the PSA and DRE, to extend the study termination date by one year (through January 2008), and to increase the number of participants by 310 to a study total of 702. **Completed in May of 2005.**

1.B. Implement combined PSA-test protocol (PSA + %fPSA) for a total of 310 African American men screened at the Summit Alta-Bates MCEPC clinic in Oakland, California (Months 4-36). The %fPSA, PSA and DRE procedures to be used are all clinical procedures now performed routinely at the study clinic (Markstein Cancer Education and Prevention Center, Alta Bates Summit Medical Center, Oakland, CA). **Initiated as of June 15, 2005; completed September 1, 2007.**

1.C. We will interview study subjects and edit dietary questionnaires for all study subjects, to include:

1.C.1. 200 participants, comprising 100 DOD-funded participants plus 100 participants funded by NIH, all to be screened during Months 3-15. **All planned NIH (100) and DOD (~100) participants were completed as of December 31, 2005.**

1.C.2. 210 additional DOD-funded participants will be screened during Months 16-36. **Completed as of September 1, 2007.**



1.C.3. For all participants to be screened in this study, previously developed dietary survey questionnaires will be used in the same manner they are being used in our ongoing corresponding NIH-funded research study (see Questionnaires, Surveys & Clinical Protocols). **Completed as of September 1, 2007.**

Task 2. Assess improvement in PC sensitivity and selectivity by addition of %fPSA test to the standard PSA test based on PSA-related test results and follow-up diagnoses in our African American study participants (Months 18-36). **Completed as of September 1, 2007.**

2.A. Data analysis methods to be used will be identical to those in use for the current related NIH-funded study (see Questionnaires, Surveys & Clinical Protocols)

2.B. Preliminary statistical analyses will be conducted during Months 18-27. **Completed as of December 31, 2006, except for %fPSA. See study publication attached.**

2.C. Final statistical analyses will be conducted during Months 27-36 **Completed as of September 1, 2007. See study abstracts attached.**

Task 3. Use Combined PSA-related test results, together with dietary survey data, to assess the potential association of HA-related exposure factors and increased PC risk in African Americans (Months 13-36). **Completed as of September 1, 2007. See study publication attached.**

3.A. Data analysis methods to be used will be identical to those in use for the current related NIH-funded study. **Analysis of initial data set (n = 392) obtained using the original protocol for NIH-funded work is described in the attached study publication. An updated analysis involving a total of 562 men was completed and was presented at the 2007 annual meeting of the American Association for Cancer Research. The updated analysis supports key study hypotheses. (See attached 2007 AACR abstract.) An updated analysis involving a total of 702 men was completed and was presented at the 2007 DOD Congressionally Mandated Medical Research Program meeting, Innovative Minds in Prostate Cancer Today, September 5-8, 2007, Atlanta,. The updated analysis supports key study hypotheses. (See attached 2007 DOD ImPACT abstract.)**

3.B. Preliminary statistical analyses of the statistical validity of Study Hypotheses 1 and 2 will occur during Months 18-27. **See study publication attached.**

3.C. Final statistical analyses of the statistical validity of Study Hypotheses 1 and 2, as well as manuscript preparation, will occur during Months 27-36. **Completed as of September 1, 2007. See study abstracts attached.**

3.D. We will also, as feasible, test the validity of Study Hypothesis 2 using combine DOD-funded data set (n = 300) with NIH-only-funded data set (n = 400), during Months 27-36. **Completed as of September 1, 2007. See study abstracts attached.**

## **Key Research Accomplishments**

The NIH-funded P01 work that set the stage for the present expanded study has accomplished the two specific aims it sought to address. We successfully applied methods for estimating HA concentrations in cooked meats based on individually expressed data on meat-specific intakes, cooking methods and doneness preferences to estimate daily PhIP intakes, and we have found these intake estimates to be positively associated with screening indicators of highly elevated PC risk in a prospective clinic-based PC screening study involving 392 African-American men in the San Francisco East Bay area. The observed positive association, which was most significant among men 55 to 70 years of age ( $p_{\text{trend}} = 0.00020$ ), remained statistically significant after adjustments for saturated fat intake, total energy intake and self-reported father/brother history of PC. These study findings were upheld in updated analyses that involved a total of 562 men (see attached 2007 AACR abstract) and 702 men (see attached 2007 DOD IMPaCT abstract) from the combined NIH- and DOD-supported work.

Future work will complete the accrual of all planned participants using the same basic study design, including the expanded screening indicator used to predict PC status (%fPSA test), and assess whether we continue to observe significant PhIP-related associations pertain to combined screening indicator data using a larger study population. In so doing, this study will continue to help define the potential value of improved screening and dietary/behavioral intervention to reduce PC risk, namely, prevention of PhIP intake by avoiding overcooked meats.

## **Reportable Outcomes**

### Abstracts/Posters

Bogen, KT, W Baker, J Chan, D Nelson, E Holly, G Keating, L Paine, and J Felton. 2005. Prostate cancer screening and dietary HA exposure in African-Americans. UCRL-POST-211476. Poster presented at the University of California Davis Health System Future Fair, May 5, 2005, UC Davis Medical Center, Sacramento, CA.

Bogen, KT, GA Keating, EA Holly, J Chan, L Paine, EL Simms, DO Nelson, and J Felton. 2005. Prostate Serum Antigen levels and dietary heterocyclic amines in

African Americans: A prospective clinic-based study. Abstract of poster accepted for presentation at the 97th Annual Meeting of the American Assoc. for Cancer Research, April 1-5, 2006, Washington, DC. UCRL-ABS-217085. Lawrence Livermore National Laboratory, Livermore, CA.

Bogen, KT, J Chan, GA Keating, LJ Paine, EL Simms, EA Holly, and JS Felton. 2006. Prostate-specific antigen levels and dietary PhIP in African Americans: A prospective clinic-based study. [Abstract] 2007 Annual Meeting of the American Association for Cancer Research, 14-18 April 2007, Los Angeles, CA. [UCRL-ABS-226551] [included in the **Appendix** of this report]

Keating, GA, KT Bogen, J Chan, LJ Paine, EL Simms EA Holly and J Felton. 2007. Elevated Prostate-Specific Antigen in African American Men with High Meat-Carcinogen Intake: a Prospective Clinic-Based Study [Abstract] Poster presentation at the DOD Congressionally Mandated Medical Research Program meeting, Innovative Minds in Prostate Cancer Today, September 5-8, 2007, Atlanta, GA. [included in the **Appendix** of this report]

### Reports and other Publications

Bogen KT. 2006. PSA-Based Screening Outcomes, Dietary Heterocyclic Amine Exposure, and Prostate Cancer Risk in African Americans: Annual Report (Year 1 of 3). UCRL-TR-218258. Lawrence Livermore National Laboratory, Livermore, CA.

Keating GA, K Bogen, and J Chan. 2007. Development of a meat frequency questionnaire for use in diet and cancer studies. *J. Am. Dietetic Assoc.* 107, 1356-62. [UCRL-JRNL-208025]

Bogen KT, GA Keating II, JM Chan, LJ Paine, EL Simms, DO Nelson, and EA Holly. 2007. Highly elevated PSA and dietary PhIP intake in a prospective clinic-based study among African Americans. *Prostate Cancer and Prostatic Diseases* 1-9. [included in the **Appendix** of this report]

Louis ED, Zheng W, Jiang W, Bogen KT, and Keating GA. 2007. Quantification of the neurotoxic  $\beta$ -carboline harmane in barbecued/grilled meat samples and correlation with level of doneness. *J. Toxicol. Environ. Health* 70, 1014-9. [UCRL-JRNL-225956] [This publication resulted from a collaboration in which a team from the Columbia University Medical School used the same LLNL meat questionnaire that also is being used in the present DOD-funded work. This publication thus represents an unexpected indirect benefit of the present DOD-funded work.]

## **Conclusions**

Prostate cancer (PC) is the second leading cause of male U.S. cancer

deaths, with African-Americans having the highest rate of PC mortality worldwide, as well as more abnormal results from screening tests that correlate with current or eventual PC. A 5-year prospective NIH-funded clinic-based study investigated whether prostate-specific antigen (PSA) and digital rectal exam (DRE) screening indicators of PC risk in 500 African-American men 50 to 70 years of age who underwent PC screening in Oakland, CA (East Bay San Francisco area), were associated with estimated dietary exposures to 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), which forms when meat is overcooked. The DOD-funded study expands that NIH-funded work by adding a new %-free-PSA test for 310 (108 from the NIH-funded study, plus 202 additional) men, results of which will be compared with PSA/ DRE results and PhIP exposures estimated by dietary interviews. For 392 men studied under the NIH protocol, an odds ratio (95% CL) of 32 (3.2, 720) for highly elevated PSA ( $\geq 20$  ng/mL) was observed in the highest 15% vs. the lower 50% of estimated daily PhIP intakes. As of 09-01-07, a total of 310 men completed participation using the expanded protocol, for a combined total of 702 men. For the final analysis of all 702 men, the corresponding OR was found to be 10 (2.9, 58). This study will help define the potential value of improved screening and dietary/behavioral intervention to reduce PC risk.

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## Appendix

Bogen, KT, J Chan, GA Keating, LJ Paine, EL Simms, EA Holly, and JS Felton. 2006. Prostate-specific antigen levels and dietary PhIP in African Americans: A prospective clinic-based study. [Abstract] 2007 Annual Meeting of the American Association for Cancer Research, 14-18 April 2007, Los Angeles, CA. [UCRL-ABS-226551]

Keating, GA, KT Bogen, J Chan, LJ Paine, EL Simms EA Holly and J Felton. 2007. Elevated Prostate-Specific Antigen in African American Men with High Meat-Carcinogen Intake: a Prospective Clinic-Based Study [Abstract] Poster presentation at the DOD Congressionally Mandated Medical Research Program meeting, Innovative Minds in Prostate Cancer Today, September 5-8, 2007, Atlanta, GA.

Bogen KT, GA Keating II, JM Chan, LJ Paine, EL Simms, DO Nelson, and EA Holly. 2007. Highly elevated PSA and dietary PhIP intake in a prospective clinic-based study among African Americans. *Prostate Cancer and Prostatic Diseases* 1-9.

[these publications follow]

## **Prostate-specific antigen levels and dietary PhIP in African Americans: A prospective clinic-based study**

Bogen, KT<sup>1</sup>, J Chan<sup>2</sup>, GA Keating<sup>1</sup>, LJ Paine<sup>3</sup>, EL Simms<sup>3</sup>, EA Holly<sup>2</sup>, and JS Felton<sup>1</sup>  
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Heterocyclic amines (HAs)—potent mutagens formed as red meat, chicken or fish cooks—cause cancer at multiple sites (including rat prostate) in rodent bioassays, and have been linked to elevated human risk of colon and other cancers. Compared to white men in the U.S., African American (AA) men have about twice the prostate cancer (PC) incidence and about twice the daily intake of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), the predominant HA in the U.S. diet. To investigate the hypothesis that dietary PhIP exposure increases PC risk, an ongoing prospective clinic-based study has compared PC screening outcomes with survey-based estimates of dietary PhIP intake by 40- to 70-year-old AA men in the Oakland, CA, area. Participants with no prior PC diagnosis, recruited through a cancer education center/screening clinic, complete food-frequency and meat cooking/consumption questionnaires, and have a prostate-specific antigen (PSA) test and digital-rectal exam. Preliminary results for 562 participants indicate that mean ( $\pm 1$  SD) daily intake of PhIP, the major HA found in cooked meats, in this group is 19 ( $\pm 24$ ) ng kg<sup>-1</sup> d<sup>-1</sup>, which is ~2-fold (and ~3-fold) greater than a national estimate of mean PhIP intake for AA (and white U.S.) men of similar age. In the present study, estimated PhIP intakes were found to be attributable mostly (65%) to chicken and positively associated ( $R^2 = 0.25$ ,  $p \sim 0$ ) with estimated saturated fat (SF) intake (a previously hypothesized environmental PC-risk factor). An odds ratio, OR, (and maximum-likelihood 95% confidence limits) of 23.6 (2.20, 533.) for PSA  $\geq 20$  ng/mL was observed for those in the highest 15% compared to the lower 50% of estimated daily PhIP intakes ( $\geq 32$  vs.  $\leq 4.8$  ng kg<sup>-1</sup> d<sup>-1</sup>), with a p-value (ptrend) of 0.0023 for a chi-square test for trend done across three (including these two) PhIP-intake groups (extended Fisher exact test p-value = 0.0063). This positive trend persisted after separate adjustments for self-reported family (brother or father) history of PC (FH), SF intake, and energy intake (ptrend = 0.0024, 0.012, and 0.0032, respectively). PSA measures were found (by Kolmogorov 2-sample tests) to be significantly higher in AA men reporting a positive FH in this study ( $p = 0.007$ ), particularly for those among the highest PSA-measure quartile in each FH group ( $p < 0.0002$ ). These preliminary results are consistent with a positive association between PhIP intake and highly elevated PSA levels, supporting the hypothesis that diet and food preparation interventions may help reduce PC risk in AA and perhaps other groups.

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## **Elevated Prostate-Specific Antigen in African American Men with High Meat-Carcinogen Intake: a Prospective Clinic-Based Study**

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Heterocyclic amines (HAs)-potent mutagens formed as red meat, chicken or fish cooks-cause cancer at multiple sites (including rat prostate) in rodent bioassays, and have been linked to elevated human risk of colon and other cancers. Compared to white men in the U.S., African American (AA) men have about twice the prostate cancer (PC) incidence and about twice the daily intake of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), the predominant HA in the U.S. diet. To investigate the hypothesis that dietary PhIP exposure increases PC risk, a clinic-based study compared PC-screening outcomes with survey-based estimates of dietary PhIP intake by 40- to 70- year-old AA men in the Oakland, CA, area. Participants with no prior PC diagnosis, recruited through a cancer education center/screening clinic, complete food-frequency and meat-cooking/intake questionnaires, and have a prostate-specific antigen (PSA) test, a determination of % free (unbound) PSA (%fPSA), and digital-rectal exam (DRE). Results for 702 participants indicate that mean ( $\pm 1$  SD) daily intake of PhIP, the major HA found in cooked meats, in this group is 22 ( $\pm 29$ ) ng kg<sup>-1</sup> d<sup>-1</sup>, which is ~2-fold (and ~3-fold) greater than a national estimate of mean PhIP intake for AA (and white U.S.) men of similar age. Estimated PhIP intakes were attributable mostly (68%) to chicken. An odds ratio, OR, (and maximum-likelihood 95% confidence limits) of 10. (2.9, 58) for men at high PC risk was observed in the highest quartile compared to the lowest half of estimated daily PhIP intakes (328.0 vs. 213.6 ng kg<sup>-1</sup> d<sup>-1</sup>) (ptrend = 0.00005), where high PC risk was defined as either a highly elevated PSA  $\geq 20$  ng/mL with %fPSA  $\leq 25\%$  or a "suspicious" DRE exam result. Interestingly, this trend was evident only in the group with self-reported family (brother or father) history of PC (extended Fisher-exact p = 0.00003), and not those without such history (ptrend = 0.44). The significant trends persisted after adjusting for SF intake or energy intake. These results are consistent with a positive association between PhIP intake and highly elevated PSA levels, supporting the hypothesis that diet and food preparation interventions may help reduce PC risk in AA and perhaps other groups.

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## ORIGINAL ARTICLE

# Highly elevated PSA and dietary PhIP intake in a prospective clinic-based study among African Americans

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African-American men die from prostate cancer (PC) nearly twice as often as white US men and consume about twice as much of the predominant US dietary heterocyclic amine, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), a genotoxic rat-prostate carcinogen found primarily in well-cooked chicken and beef. To investigate the hypothesis that PhIP exposure increases PC risk, an ongoing prospective clinic-based study compared PC screening outcomes with survey-based estimates of dietary PhIP intake among 40–70-year-old African-American men with no prior PC in Oakland, CA. They completed food-frequency and meat-cooking/consumption questionnaires and had a prostate-specific antigen (PSA) test and digital-rectal exam. Results for 392 men indicated a 17 ( $\pm 17$ ) ng/kg day mean ( $\pm 1$  s.d.) daily intake of PhIP, about twice that of white US men of similar age. PhIP intake was attributable mostly to chicken (61%) and positively associated ( $R^2 = 0.32$ ,  $P < 0.0001$ ) with saturated fat intake. An odds ratio (95% confidence interval) of 31 (3.1–690) for highly elevated PSA  $\geq 20$  ng/ml was observed in the highest 15% vs lowest 50% of estimated daily PhIP intake ( $\geq 30$  vs  $\leq 10$  ng/kg day) among men 50+ years old ( $P = 0.0002$  for trend) and remained significant after adjustment for self-reported family history of (brother or father) PC, saturated fat intake and total energy intake. PSA measures were higher in African-American men with positive family history ( $P = 0.007$  all men,  $P < 0.0001$  highest PSA quartile). These preliminary results are consistent with a positive association between PhIP intake and highly elevated PSA, supporting the hypothesis that dietary intervention may help reduce PC risk.

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**Keywords:** cooked meat; diet; energy; heterocyclic amines; mutagens; race

## Introduction

Heterocyclic amines (HAs) are potent mutagens formed in meats, chicken and fish as it is cooked to higher-doneness levels by heat-intensive cooking methods.<sup>1–3</sup> HAs also cause cancer in a variety of tissue types in multiple bioassays in different animal species, strains and sexes, and so may present a human dietary cancer risk.<sup>4,5</sup> A predominant HA found particularly in well-done chicken and beef is 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP).<sup>6–11</sup> Dietary exposure to PhIP has been shown to induce colon, intestinal and mammary adenocarcinomas in rats<sup>12–18</sup> and prostate cancer in rats.<sup>19,20</sup> HAs including PhIP are positive in numerous genotoxicity assays.<sup>21,22</sup> Like other HAs, PhIP is metabolically activated by P450 and N-acetyltransferase enzymes to genotoxic metabolites, which in the case of PhIP mutate both rat and human prostate DNA.<sup>21,23–40</sup>

PhIP also has potent estrogenic activity that may act synergistically with genotoxic effects to enhance its carcinogenicity.<sup>41</sup>

In the US, prostate cancer (PC) is a leading cause of cancer death among men, with African Americans having the highest age-specific prostate cancer rate in the world, and a >2-fold higher rate of mortality for PC than white men in the US.<sup>42–44</sup> Although family history of PC (particularly father/brother) is clearly linked to substantially elevated PC risk<sup>45–48</sup> and a human-PC-specific chromosome translocation has been identified,<sup>49</sup> there is no evidence for a predominant heritable factor for PC. Racial-ethnic differences in testosterone-related hormone and/or receptor levels may also affect racial-ethnic differences in PC risk, but their role remains unclear.<sup>50–52</sup>

Substantial geographical variations in PC incidence indicate the importance of one or more dietary or other environmental factors.<sup>53–55</sup> Dietary intake of animal or saturated fat often has been linked to increased PC risk in previous studies, including among African-American men. However, these associations appear too weak to explain more than a small fraction of observed racial-ethnic differences in PC risk.<sup>56–59</sup> This also

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appears to be the case for other environmental/dietary factors examined such as calcium, cruciferous vegetables, vitamin D, UV from sunlight, lycopene and body size.<sup>60–66</sup> Because cooked-meat intake is positively associated with total saturated fat intake,<sup>67</sup> previous studies that focused on animal or saturated fat *per se* may have estimated effects due largely or entirely to meat-related HA intake.

Consumption of cooked meats and associated HAs has been linked to increased risks of colorectal adenoma/adenocarcinoma and of cancer of the stomach, breast, lung and prostate,<sup>68–90</sup> whereas fewer studies have reported no such associations.<sup>89,91–94</sup> One study<sup>94</sup> quantified HA-specific intake estimates (including for PhIP), but detected no positive association of consumption of any HA (from white or red meat) with elevated risk of breast cancer, regardless of *N*-acetyltransferase genotype. Other positive studies have tended to include those with HA exposure quantified after adjusting for factors expected to determine HA intake, namely, meat type, consumption frequency, cooking method and cooking doneness. For example, recently published results from a study that used the Block Brief 2000 survey to assess meat-related exposures detected elevated PC risk among 692 African-American men (but not among nearly 65 000 white men) who consumed relatively more cooked red or processed meats.<sup>89</sup>

Periodic screening for serum levels of prostate-specific antigen (PSA) is useful to detect early PC and benign prostatic hyperplasia (BPH) because PSA levels in serum are positively associated with age, prostate volume and prostatic neoplastic disease.<sup>95–97</sup> Significantly higher PSA levels are found in African Americans than in white subjects, even after adjustment for age and prostate volume in men without PC, and for PC grade and stage in men with PC.<sup>98–100</sup> This is due to greater PSA secretion per unit prostate volume by African-American men.<sup>101</sup> Although a substantial fraction of 'slightly' elevated PSA levels (between 4 and 10 ng/ml) is attributable to BPH or infection, 'highly elevated' PSA levels ( $\geq 20$  ng/ml) typically indicate a strong likelihood of localized or metastatic PC, with  $>99\%$  specificity<sup>102–104</sup> and with  $>95\%$  positive predictivity for men of age 50–69 years.<sup>105</sup> Likewise, even among men with a PSA measure  $<4.0$  ng/ml (often considered within the 'normal' range), it is estimated that about 15% later may be diagnosed with PC.<sup>106</sup>

To investigate further whether PhIP intake is related to PC risk, we conducted a study to assess the association between highly elevated PSA as a screening indicator for elevated PC risk and estimated dietary exposure to PhIP among African-American men.

## Methods

### *Study design and participants*

In an ongoing clinic-based prospective study using Institutional Review Board-approved human participant protocols, we enrolled 392 African-American men from the Oakland, CA area using the following inclusion criteria: (1) African-American men between 40 and 70 years old; (2) no previous PC diagnosis or contra-indicated medical condition; and (3) written informed

consent. Participation was enhanced by a \$30 incentive payment and by  $>10$  years of previous PC-related community outreach undertaken by the study clinic (the Markstein Cancer Education and Prevention Center at Alta Bates Summit Medical Center in Oakland, CA). After providing written informed consent, each participant completed a PC screening medical questionnaire, answered general and detailed meat-related dietary questions and was provided free PC screening including a PSA blood test and a digital-rectal exam (DRE) by a board-certified urologist.

Oakland is a metropolitan city near San Francisco within California's Alameda County, in which prostate cancer is the most common cancer in African-American men with about 920 new cases predicted in 2006.<sup>107</sup> In 2004, Alameda County had a 14.1% African-American population, whereas in Oakland, the site of the medical center where our study was conducted, 35.7% of the population was African American, and African-American populations statewide and nationwide in 2004 were 6.8 and 12.8%, respectively.<sup>108</sup> African-American men in the Alameda County recruitment area have a median age of 34 years, median per capita income of about \$34,700 and a currently married rate of 39%.<sup>108</sup> Outreach was tailored to African-American men aged 40–70 in Alameda County reflecting its current socio-economically diverse African-American population, including a large fraction of unmarried men who do not benefit from positive spousal influences on health-care choices. Outreach was conducted at more sites frequented by men including barbershops catering to African Americans, social service organizations, African-American churches, senior centers and housing units, Veterans Administration outpatient clinics, community health clinics and medical practices of members of the Sinkler-Miller medical association, a professional association of African-American physicians.

### *Dietary assessment*

General dietary intake over the previous year was estimated using the Block-2000 food-frequency questionnaire (FFQ) with portion-size standardized food-model photographs to help each participant select portion sizes.<sup>109</sup> Dietary data on specific meats consumed and preferred cooking methods over the previous year were obtained using an additional questionnaire that includes a validated set of standard meat-doneness descriptors and corresponding set of meat-doneness photographs.<sup>110–112</sup> All dietary questionnaire data were obtained by in-person interviews administered by trained dietary interviewers.

### *Data analysis*

Combined survey data were used as previously described<sup>113–115</sup> to estimate annual average dietary PhIP intake from all sources by each participant. Total and basal energy intake in kcal per kg body weight was estimated for each study participant using previously described methods.<sup>113</sup> Standard methods were used to assess the significance of linear associations and outliers where noted, and to assess Pearson product-moment correlations.<sup>116,117</sup> Approximate significance of differences in mean HA intake was compared using

Welch's *t*-test whereas unequal variance was assessed by corresponding F-tests. Differences in PSA by family history were assessed by the Wilcoxon test.<sup>118</sup> Odds ratio (OR) and corresponding 95% confidence interval (CI) estimates obtained by numerical maximum-likelihood procedures are reported together with corresponding  $\chi^2$  tests for trend (with or without adjustment for specified factors).<sup>119</sup> Reported Fisher's exact *P*-values are for two-tailed tests. Significance *P*-values  $\leq 10^{-10}$  were reported as being approximately 0, and values  $< 0.10$  were reported to one significant figure. All calculations were carried out using *Mathematica* 5.2 software.<sup>120</sup>

## Results

### PhIP intake

Data on 392 African-American men who participated in this study are summarized in Table 1. Corresponding estimated average daily intake of specific meats and total PhIP is summarized in Table 2. The empirical distribution of estimated daily intake of PhIP from all meats (total PhIP) had geometric and arithmetic mean values of 9.6 and 17 ng/kg/day, respectively, and a geometric standard deviation (s.d.) of 3.3. A total of 89% of inter-individual variance in PhIP intake could be explained by intake of specific meats and the cooking method of those meats, and doneness-preference data appeared to explain the remaining 11% of inter-individual variation in PhIP intake. Ratios of total to basal daily intake rates of energy per unit body weight estimated for this study population had an arithmetic mean ( $\pm 1$  standard error of the mean (s.e.m.)) of 1.57 ( $\pm 0.046$ ), not significantly

different ( $P = 0.51$ ) from the value of 1.6 expected for reference adult men.<sup>113</sup>

### Correlation between PhIP and other dietary measures

Estimated daily intake of total PhIP explained approximately 32% of observed inter-individual variance in corresponding estimated intake of saturated fat per unit body weight (Figure 1). Similar or greater levels of positive correlation were observed between estimated total PhIP intake and energy intake ratio ( $E_{\text{food}}:E_{\text{basal}}$ ) ( $R^2 = 0.26$ ), total energy intake ( $E_{\text{food}}$ ) ( $R^2 = 0.27$ ) and total meat intake (g/kg/day) ( $R^2 = 0.68$ ). There were strong correlations between estimated intake of total energy and saturated fat ( $R^2 = 0.84$ ) and between total energy and  $E_{\text{food}}:E_{\text{basal}}$  ( $R^2 = 0.97$ ).

### PSA, age and family history

PSA measures were weakly positively associated with participant age and attained statistical significance for all measures  $< 4$  ng/ml ( $R^2 = 0.051$ ,  $P = 0.00001$ ), but not for

**Table 2** Summary of meat-specific PhIP intake for 392 study participants in an Oakland, CA study on diet and prostate screening

	Meat type					
	Chicken	Burger	Beef	Pork	Fish	Bacon
Mean <sup>a</sup>	10.3	2.5	2.4	0.3	0.4	<0.1
s.d. <sup>a</sup>	13.0	3.7	4.3	0.6	1.0	0.1
s.d.m. <sup>a</sup>	0.6	0.2	0.2	0.03	0.05	0.01
CVM%	6.2	7.4	9.1	8.2	15.0	30.0
%all	61.0	15.0	14.0	2.2	2.2	0.1

Abbreviations: s.d., standard deviation; s.d.m., standard deviation of the mean; CVM% =  $100\% \times (\text{s.d.m.}/\text{mean})$

<sup>a</sup>Values listed are in ng/kg/day; arithmetic mean values are shown to a value of 0.1 ng/kg/day. Meats listed exclude those rarely used or meats that account for relatively little HA, such as lamb and organ meats. The meat components of food mixtures were excluded from those listed individually, but are included in the 'All' category.

**Table 1** Baseline characteristics of 392 participants in an Oakland, CA study on diet and prostate cancer screening

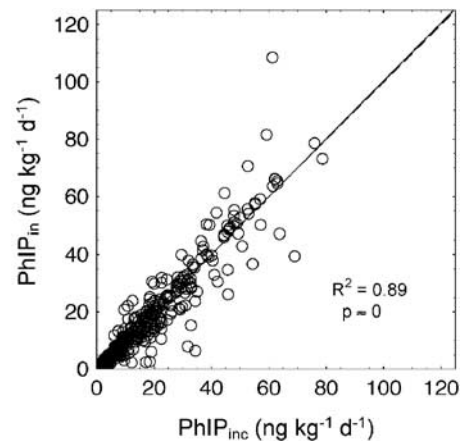
Variable <sup>a</sup>	Value(s)	n	Mean
Age (years)	39–50	115	47
	51–60	216	55
	61–70	61	64
	All	392	54
Weight (kg)	54–163	392	86 <sup>a</sup>
BMI (kg/cm <sup>2</sup> )	<30	317	25
	$\geq 30$	75	34
	18–46	392	27
PSA (ng/ml)	<2	328	0.79
	2 to <4	42	3
	4 to <10	10	6
	10 to <20	7	14
	$\geq 20$	5	47
		PSA < 4	PSA $\geq 4$ <sup>b</sup>
DRE	Normal <sup>b</sup>	233	15
	BPH <sup>b</sup>	108	4
	Suspicious <sup>b</sup>	12	3
Family history <sup>c</sup>	No	333	15
	Yes	37	7

Abbreviations: BMI, body mass index; BPH, benign prostatic hyperplasia; DRE, digital-rectal exam; PSA, prostate-specific antigen.

<sup>a</sup>Weight median (interquartile range) = 84 (75–95) kg.

<sup>b</sup>Normal = no urinary, BPH or other symptoms; suspicious = abnormal DRE result leading to medical referral. Each abnormal PSA result ( $\geq 4.0$  ng/ml) triggered a medical follow-up referral.

<sup>c</sup>Family history, self-reported brother(s) and/or father diagnosed with prostate cancer. Association of positive family history with PSA  $\geq 4$  ng/ml; Fisher's exact test ( $P = 0.007$ ).



**Figure 1** PhIP intake estimated using individual-level doneness-preference data (Y axis,  $\text{PhIP}_{\text{in}}$ ), compared with intake estimated using all individuals combined with their meat- and cooking-method-specific average levels of preferred doneness (X axis,  $\text{PhIP}_{\text{inc}}$ ). The linear fit of the data shown (dashed line) has an estimated (and corresponding 95% CI) intercept of 0.58 ( $-1.4$ – $0.23$ ) and slope of 1.01 ( $0.974$ – $1.045$ ); an identity relation (solid line) is shown for comparison.

all measures  $\geq 4$  ng/ml ( $R^2 = 0.035$ ,  $P = 0.41$ ). PSA measures  $\geq 20$  ng/ml were observed only among older participants, aged 55–65 years. PSA measures among participants reporting vs not reporting a father and/or brother with PC were greater among those reporting such a family history ( $P = 0.007$  by Wilcoxon test), particularly when the comparison was restricted to the upper quartile of PSA measures in each family history category ( $P = 0.0006$  by Wilcoxon test). Positive family history also was positively associated with elevated PSA defined as  $\geq 4$  ng/ml ( $P = 0.007$ ; Table 1).

### PhIP and PSA

As noted, the above-estimated PhIP intake had a highly skewed distribution; so association between PhIP intake and highly elevated PSA ( $\geq 20$  ng/ml) status was investigated using PhIP intake bin boundaries defined by the 50th, 70th and 85th percentile values of the empirical intake distribution. PhIP intake level was positively associated with highly elevated PSA status, when comparing those in the highest 15% vs the lowest 50% of PhIP consumption, with or without single-variable adjustment for father/brother family history of PC, saturated fat intake or total energy (corresponding  $P$ -values for trend:  $P_{\text{trend}} = 0.01$  for saturated fat adjust-

ment,  $P_{\text{trend}} \leq 0.003$  for all other tests) (Table 3). All highly elevated PhIP measures occurred in men within a fairly narrow age range (55–65 years old). In analyses limited to men aged  $\geq 50$  years, men in the highest 15% compared with those in the bottom 50% of estimated PhIP intake had a conditional maximum-likelihood odds ratio of 31 (95% CI: 3.1–690) for having a highly elevated PSA ( $P_{\text{trend}} = 0.0002$ ). For this age group, adjustment for family history of PC, saturated fat intake or total energy yielded identical OR estimates and only slightly greater  $P_{\text{trend}}$  estimates (data not shown).

A similar pattern of positive association was observed between estimated PhIP intake and men with highly elevated PC risk defined as either PSA  $\geq 20$  ng/ml or a 'suspicious' abnormal DRE result leading to medical referral (data not shown). As expected, men with mildly elevated PSA ( $\geq 4.0$  ng/ml) were about four times more likely to have had a suspicious DRE than did participants with PSA  $< 4.0$  ng/ml, although this difference did not rule out chance ( $P = 0.07$ , Fisher's exact test). Suspicious DRE results were not obtained for any participant with a PSA measure  $\geq 20$  ng/ml. PSA level categorized either in quintiles or 50th, 70th, 85th percentile intervals was not associated with saturated fat intake, total energy intake or body mass index, with or without adjustment for PhIP intake ( $P_{\text{trend}} > 0.10$  for each).

**Table 3** Association of PhIP intake with elevated prostate-specific antigen  $\geq 20$  ng/ml among 392 African-American men, Oakland, CA

Adjustment or stratification <sup>a</sup>		PSA ≥ 20 ng/ml <sup>b</sup>				
Average PhIP intake <sup>a</sup> PR	(ng kg <sup>-1</sup> day <sup>-1</sup> )	m	n	OR	95% CI	P <sub>trend</sub>
All data						
0–50	4.8	0	196	1		
> 50–70	14.6	1	78	7.6	—	
> 70–85	25.5	1	59	10.0	—	
> 85–100	49.7	3	59	24.0	2.2–540	0.002
Adj. for FH						
(same as above)		(m, n, ORs, LCLs and UCLs same as for all data)				0.003
FH–						
0–50	4.8	0	174	1		
> 50–70	14.6	1	69	7.6	—	
> 70–85	25.5	1	53	9.8	—	
> 85–100	49.7	1	52	10.0	—	0.10
FH+						
0–50	4.8	0	22	1		
> 50–70	14.6	0	9	—	—	
> 70–85	25.5	0	6	—	—	
> 85–100	49.7	2	7	18.0	1.4–540	0.002
Adj. for SatFat						
0–50:	4.8	0	196	1		
> 50–70	14.6	1	78	3.4	—	
> 70–85	25.5	1	59	4.1	—	
> 85–100	49.7	3	59	24.0	2.2–540	0.01
Adj. for kcal						
0–50	4.8	0	196	1		
> 50–70	14.6	1	78	14.	—	
> 70–85	25.5	1	59	4.3	—	
> 85–100	49.7	3	59	24.0	2.2–540	0.003

Abbreviations: CI, confidence interval (two-tailed), omitted if interval includes 1; FH, family history of prostate cancer, self-reported father or brother diagnosed; kcal, total energy intake per kg body weight; OR, maximum likelihood odds ratio estimate; PR, percentile range (PR and corresponding mean PhIP intake values pertain to all 392 participants combined);  $P_{\text{trend}} = P$ -value for  $\chi^2$  test of linear, or (as indicated) adjusted linear, trend.

<sup>a</sup>Trend analyses adjusting for Saturated fat or kcal were each performed using the adjustment variable dichotomized at its median value. All men were  $\leq 70$  years old.

<sup>b</sup>m = number with PSA  $\geq 20$  ng/ml among total n participants included in the analysis.



## Discussion

These interim data are consistent with the hypothesis that estimated dietary exposure to PhIP is related to screening indicators of PC risk such as elevated PSA levels or suspicious DRE results among African-American men. Although this conclusion remains preliminary owing to the small number of men in this prospective study to date, it is supported by the consistency of the pattern of results observed and their level of statistical significance. We observed a positive association between elevated PSA and a father/brother history of PC, which is consistent with other studies in the literature that have linked such history to elevated PC risk,<sup>45–48</sup> and reflects the integrity of the study design.

A potential specific link between PhIP intake and the elevated risk of PC experienced by African-American compared with Caucasian men is suggested by relatively greater levels of PhIP and its metabolites detected in urine sampled from the two groups.<sup>121</sup> However, urine analyses only provide a measure of PhIP exposure within 12–24 h before sampling.<sup>122</sup> Such a link is supported by studies using meat- and cooking-method-specific HA concentration estimates from multi-laboratory sets of experimental cooking data.<sup>113–115</sup> These studies estimated intake of PhIP and other HAs among >20 000 US participants (including >3000 African Americans)<sup>123</sup> in the nationwide, stratified, random-sample US Continuing Survey of Food Intake by Individuals (CSFII).<sup>124</sup> Analyzed by age, sex and race/ethnicity, these HA exposure assessments showed that African-American men consumed on average at least twice (and boys through age 15 about three times) as much PhIP (and total HA) per kg body weight per day as did corresponding US Caucasians, and that for both groups there was a small, positive correlation between estimated mean intake of PhIP and of saturated or total fat.<sup>113,114</sup>

No PhIP-related PC associations were reported in a study of 317 PC cases and 480 age-matched controls that compared HA intake (primarily from cooked lamb) and PC risk in New Zealand men.<sup>78</sup> They did report an association between PC risk and consumption of beefsteak by the higher cooking-doneness category (OR (95% CI) = 1.8 (1.1–3.0), two-sided  $P_{\text{trend}} = 0.008$ ). The relevance of this finding to potential associations between PC and dietary HA or PhIP intake in the US for African-American men is unknown. The incidence of PC in New Zealand is only half that of Caucasian men in the US.<sup>44</sup> Average meat (primarily lamb) consumption by participants (~150 g/day) was well below that of US Caucasian men (260 g/day) and even further below that of African-American men (304 g/day) (Table 10A in US Department of Agriculture (USDA)<sup>67</sup>). Moreover, the estimated minimum PhIP intake (224 ng/day) in the *top* exposure quartile of the New Zealand men<sup>78</sup> was well below the estimated average PhIP intake by Caucasian men (390 ng/day) and that of African-American men (600 ng/day).<sup>113</sup> This difference reflects dissimilar meat consumption and cooking patterns in these two groups. Assuming a linear dose-response, extrapolation from elevated OR estimates  $\geq 2.2$  obtained in the present study down to PhIP intakes as low as those in the New Zealand study would require that study to have detected an OR as low as ~1.2 with 95% CI at 80% statistical

power, whereas only OR values  $\geq 1.6$  were detectable in that study.<sup>124</sup> Alternatively, the lack of a PhIP-related association with PC risk in the New Zealand case-control study could reflect that men from that study (or perhaps all Caucasian men) are, for genetic and/or environmental reasons, less susceptible to the effects of this risk factor than are the African-American participants of our clinic-based study.

Ideally, a prospective study accumulates good diagnostic data and data on exposure- or treatment-related variables of interest. One key limitation of this study is that, despite ongoing work to obtain corresponding follow-up diagnostic data, a PC diagnosis is not yet available for all participants who received either positive or highly elevated PC screening results or who received a 'suspicious' DRE leading to medical referral. Compared to a case-control design, the prospective design used in this ongoing study has the advantage of being double blind, insofar as PC screening results are not known by the participant or by study investigators until after each participant has provided dietary survey data. This design eliminates potential bias in participants' self-reported cooking preferences that may be associated with knowledge of PC screening results or PC status. This is important in view of evidence that prior knowledge of cancer-related status may affect dietary recall and so induce significant differential misclassification.<sup>125</sup>

A second key limitation was the lack of any biomarker generally considered reliable enough to assess chronic PhIP exposures. This required our study, like previous similar studies of HA-related cancer risks, to rely instead on FFQ data. Despite their tendency to slightly underestimate intakes, relative to estimates made using more accurate but also more inconvenient and expensive multiple 24 h food diaries, FFQs have been widely used as the method of choice to assess long-term nutritional exposures and to estimate specific disease risks in relation to such exposures.<sup>126</sup> Data from self-administered Block dietary FFQs, in particular, were shown to be effective at estimating intakes of each of four major meat-related nutrients (protein, total fat, saturated fat and cholesterol) to within <15% of corresponding estimates obtained using 24 h recall diaries among 226 men who participated in the 1997–1998 'Eating at America's Table' study.<sup>127</sup> A self-administered meat-specific FFQ including questions on doneness and cooking method preferences (similar to the meat-specific FFQ used in the present study) was more recently compared directly to data from 24 h recall diaries.<sup>112</sup> In that study, the FFQ method was shown to underestimate PhIP intakes, but also to classify >60% of individual intakes of each of two HAs (including PhIP) to the same or adjacent quintiles, and to correctly classify lowest vs highest quintiles for >94% of such intakes.<sup>112</sup> That study concluded that observed levels of FFQ-associated PhIP intake misclassification would tend to cause underestimation of true relative risks associated with increased PhIP intake estimated from self-administered FFQ data.<sup>112</sup> Rates of misclassification of PhIP and other dietary factors were likely reduced in the present study because our FFQs were administered in person by trained interviewers.

In conclusion, we applied methods to estimate HA concentrations in cooked meats based on individually expressed data on meat-specific intake, cooking method and doneness preference to estimate daily PhIP intake.

We observed these intake estimates to be positively associated with screening indicators of highly elevated PC risk in a prospective clinic-based PC screening study with nearly 400 African-American men in the East Bay Oakland, CA area. The observed positive association, even more significant among men 50–70 years of age, remained statistically significant after adjustments for saturated fat intake, total energy intake and self-reported father/brother history of PC. We will continue to accrue participants in this study, expand the screening indicators used to predict PC status and assess whether the observed PhIP-related association pertains to incident PC disease.

## Abbreviations

HA, heterocyclic amine; PC, prostate cancer; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (105650-23-5).

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